Volume 209, number 2 FEBS 4272 December 1986

Point mutations of human interleukin-1 with decreased receptor binding affinity

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Received 1 October 1986

Interleukin-1 (IL-1) is a monocyte-derived polypeptide hormone that interacts with a plasma membrane receptor. We have used oligonucleotide-directed mutagenesis to construct mutant human IL-1 proteins. Three different point mutants in a unique histidine residue (position 30) exhibited varying degrees of reduced IL-1 receptor binding affinity, whereas point mutants at five other residues behaved normally. Structural analysis of these mutant proteins by nuclear magnetic resonance spectroscopy detected no (or only minor) conformational changes relative to wild-type IL-1. These data suggest that the unique histidine residue influences the architecture of the receptor binding site on human IL-1.

Interleukin-1 *Site-directed mutagenesis* *Interleukin-1 receptor* *NMR*

1. INTRODUCTION

Interleukin-1 (IL-1) refers to a family of monocyte-derived polypeptide hormones displaying a wide range of biological activities including fever induction and augmentation of lymphocyte proliferation (reviews [1,2]). Recent cDNA cloning studies [3–5] have shown that IL-1 bioactivity can be mediated by 2 distinct 17 kDa molecules (IL-1α and IL-1β) each of which is produced by cleavage of a 31 kDa precursor. The existence of plasma membrane receptors for IL-1 has been demonstrated by direct radiolabeled ligand binding studies [6–9] and the putative receptor has been recently identified by chemical cross-linking [6,7,10]. Despite their limited homology at the amino acid level (23%), it is now clear that IL-1α and IL-1β both compete for binding to the same receptor [7–10]. It is therefore of interest to determine the structural domains of the IL-1α and IL-1β molecules that are involved in ligand binding. Towards this end, we have used recombinant DNA technology to derive a series of point mutations in the IL-1β molecule. In this communication, we show that three different substitutions of the unique histidine residue (position 30) result in mutant IL-1β molecules exhibiting varying degrees of reduced binding affinity for the IL-1 receptor, whereas five substitutions at other amino acid residues have no effect. Analysis of these mutant proteins by nuclear magnetic resonance (NMR) spectroscopy further indicates that the His_{30} substitutions do not detectably alter the overall conformation of the IL-1β molecule. These data raise the possibility that this unique histidine residue may contribute directly or indirectly to the molecular architecture of the receptor binding site on IL-1β.

2. MATERIALS AND METHODS

2.1. *Cells*

A variant subline of murine EL4 thymoma cells

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

(designated EL4-6.1) was derived by mutagenesis and drug selection as described elsewhere [11].

2.2. *Preparation of IL-1β mutants*

The procedure for introducing point mutations in the IL-1β molecule via site directed mutagenesis has been described elsewhere [12]. Mutant proteins were purified as described for the wild type protein [13].

2.3. *NMR spectroscopy*

Samples for NMR spectroscopy contained 1.4 to 1.6 mM protein in D_2O/sodium phosphate buffer (160–300 mM), sodium azide (3 mM), pH 7.5. All spectra were recorded on a Bruker AM 500 spectrometer equipped with an Aspect 3000 computer at 25°C using a 90° observation pulse and employing a 2 s relaxation delay. 64 transients obtained by quadrature detection with 8192 data points and a spectral width of 8.2 kHz were averaged for each spectrum. Prior to Fourier transformation the free induction decay was multiplied by an exponential equivalent to a line broadening of 1 Hz. Chemical shifts are quoted relative to 4,4-dimethylsilapentane-1-sulfonate (DSS).

2.4. *Labeling of IL-1 and receptor binding assay*

Recombinant human IL-1α was radioiodinated according to the chloramine T method as described in detail elsewhere [9]. The radiolabeled ligand (^{125}I-IL-1α) retained full biological activity as measured by its ability to induce the secretion of interleukin-2 (IL-2) in EL4-6.1 cells as well as in the thymocyte IL-1 assay. The specific activity of ^{125}I-IL-1 was 10^6 cpm/ng.

Binding of ^{125}I-IL-1 to EL4-6.1 cells was carried out according to Dower et al. [6] with minor modifications [9]. Briefly, aliquots of 5×10^5 EL4-6.1 cells in 200 μl final volume were incubated with ^{125}I-IL-1α for 3–4 h at 4°C to allow equilibrium binding. Bound and free radioactivity were then separated by centrifugation through an oil gradient. For competition binding experiments, various concentrations of unlabeled native (or mutant) IL-1β molecules were mixed with a fixed concentration (usually 1 ng/ml) of ^{125}I-IL-1 prior to addition of EL4-6.1 cells. Inhibition of ^{125}I-IL-1α binding was calculated as a percentage relative to binding in the absence of cold competitor (normally 4000–7000 cpm).

3. RESULTS AND DISCUSSION

The various point mutations of the human IL-1β molecule used in this study are indicated in table 1. In order to test the effect of these substitutions on the ability of the mutant IL-1 molecules to bind to the IL-1 receptor, we made use of a recently described variant subline of EL4 thymoma cells (designated EL4-6.1) that responds in vitro to IL-1 [14] and expresses large numbers (~20000 per cell) of membrane IL-1 receptors [9]. As indicated in fig.1A, amino acid substitutions at positions 24, 68, 71, 90 or 120/121 had no demonstrable effect on IL-1 binding in that the mutant molecules competed as well as intact IL-1β for inhibition of ^{125}I-IL-1α binding. (^{125}I-IL-1α was used for these studies since it could be radioiodinated without loss of biological activity [9] and since it competes for binding with IL-1β.) In contrast, 3 different substitutions at the unique histidine residue (position 30 in the mature IL-1β molecule) resulted in varying degrees of loss of binding activity (fig.1B). Thus the replacement of His_{30} by Gln, Asn, or Arg led to reductions of approx. 2-fold, 30-fold, or 100-fold, respectively, in competitive IL-1 binding activity.

The reduced receptor binding affinity of the histidine substitution mutants could imply that this residue contributes to the IL-1 receptor binding site on IL-1β. Alternatively, these substitutions could result in major changes in IL-1β conformation. To address the latter issue, NMR spec-

Table 1

IL-1 point mutations used in this study

Amino acid residue[a]	Substitution		Designation
	Wild type	Mutant	
24	Tyr	Phe	1
30	His	Gln	2
30	His	Asn	3
30	His	Arg	4
68	Tyr	Phe	5
71	Cys	Ser	6
90	Tyr	Phe	7
120/121	Trp/Tyr	Phe/Phe	8[b]

[a] Refers to mature form of IL-1 molecule

[b] Mutant 8 has 2 contiguous amino acid substitutions

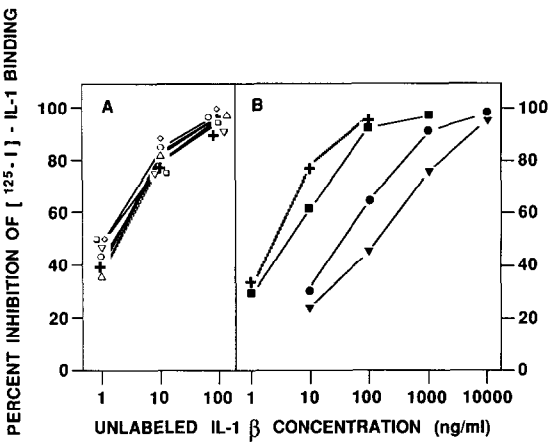

Fig.1. Competition binding analysis of IL-1β mutants. The indicated concentrations of unlabeled mutant (or control wild type) IL-1β were mixed at 4°C with ^{125}I-IL-1α (1 ng/ml) prior to addition of EL4-6.1 cells (5×10^5/tube). After 4 h at 4°C, bound radioactivity was evaluated by centrifugation of the cells through an oil gradient. Data are expressed as percent inhibition of ^{125}I-IL-1 binding as compared to untreated controls. (A) Mutants 1 (○), 5 (△), 6 (◇), 7 (▽) and 8 (□) are compared to wild type (+). (B) His mutants 2 (■), 3 (●) and 4 (▼) are compared to wild type (+). Data in panel B represent mean values from 4 independent experiments. For convenience, mutants are numbered as in table 1.

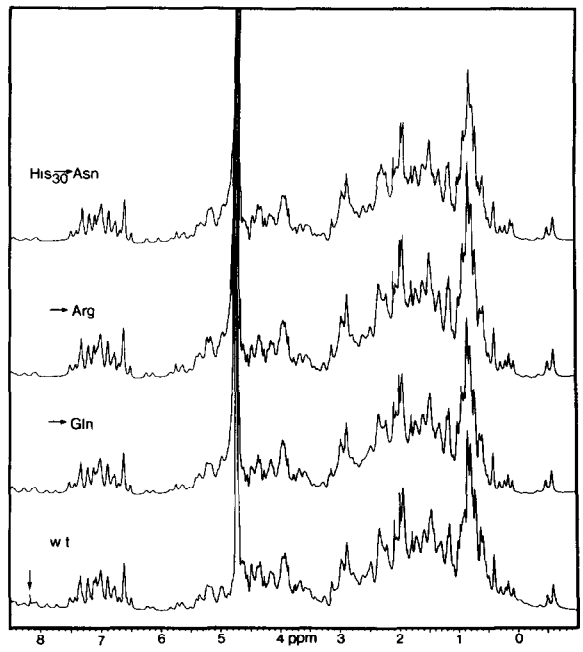

Fig.2. 500 MHz ^{1}H-NMR spectra of wild type IL-1β and histidine substitution mutants at 25°C. Arrow indicates position of histidine resonance in wild type spectrum.

troscopy was performed on the three His_{30} substitution mutants as well as the native IL-1β protein. As shown in fig.2, no detectable differences in the 500 MHz proton NMR spectra were seen with the exception of the expected disappearance of His resonances in the aromatic region of the mutant spectra (8.19 and 7.12 ppm). Using two-dimensional homonuclear Hartmann-Hahn spectroscopy, similar through-bond cross-peaks were observed for IL-1β and the three histidine mutants (not shown; see [12] for spectrum of His⟶Asn mutant). In studies to be reported elsewhere [12], the other five substitution mutants investigated in this study were likewise found not to differ dramatically from native IL-1β in their NMR spectra, although the Tyr_{90} mutant showed the most distinct changes.

Since proton NMR spectra are extremely sensitive to conformational changes in proteins [15], the spectral identity of IL-1β and the three His_{30} substitution mutants essentially rules out major structural alterations as an explanation for the reduced binding affinity of the mutants. We therefore favor the hypothesis that His_{30} may contribute in some way to the receptor binding site on the IL-1β molecule. In this context, we have previously shown [12] that this residue has an unusually high pK_a (7.5) compared to free histidine or solvent accessible histidine in proteins (pK_a ~6.5). Since a histidine residue with a high pK_a is most likely involved in hydrogen bonding or salt bridge formation, we propose that His_{30} is responsible for a particular local protein conformation which in turn is important for IL-1 receptor binding. According to such a model, the hierarchy in receptor binding affinities exhibited by the three histidine substitution mutants (Gln > Asn > Arg) could be explained on the basis of steric compatibility, since the NH_2 group of glutamine could theoretically substitute more readily for the imidazole side chain of histidine in hydrogen bonding, whereas asparagine and arginine side groups would be too short or long, respectively.

Previous studies [16] have indicated that certain

proteolytic fragments of the IL-1β molecule retain biological activity (as measured by induction of T lymphocyte proliferation), although the amino acid sequence (and consequent molecular localization) of these fragments was not reported. More recently, Rosenwasser et al. [17] have described the expression of biologically active truncated IL-1β molecules in transfected COS cells. Using this approach, a peptide of 62 amino acids (corresponding to positions 20–81 of the mature IL-1β molecule) retained detectable biological activity as measured by the induction of proliferation of thymocytes or cloned T helper cells. Significantly, this biologically active IL-1 peptide includes the His_{30} residue that we postulate to be important in receptor binding.

Amino acid sequence analysis of human IL-1α and IL-1β indicates that these proteins share only 23% homology [5]. Nevertheless, competition binding studies [7–10], including those described in this report, demonstrate directly that the α and β forms of human IL-1 bind with comparable affinity to the same plasma membrane receptor. In searching for a putative common receptor binding site on IL-1α and IL-1β, it is interesting to note that one of the three histidine residues of IL-1α, like His_{30} of IL-1β, has an unusually high pK_a (7.5), whereas the two other histidine residues in IL-1α titrate with pK_a values in the normal range. These results, while clearly indirect, raise the possibility that homologous IL-1α and IL-1β receptor binding sites may exist in the vicinity of these structurally homologous His residues. Direct testing of this hypothesis via mutation of the His residues of IL-1α is currently in progress.

ACKNOWLEDGEMENTS

We wish to thank Anne-Lise Peitrequin and Françoise Besençon for expert technical assistance.

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